Genetic recombination: **Helicases and topoisomerases link up**

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RecQ helicases and topoisomerase III are both required for genome stability, particularly to prevent 'promiscuous' genetic recombination. A recent study demonstrates that, together, these enzymes can catalyse the interlinking of plasmid DNA, and suggests a novel mechanism for the control of recombination.

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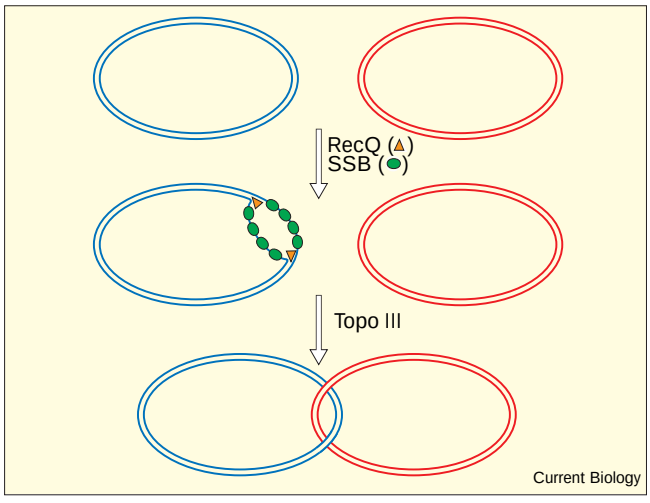
The maintenance of genome stability is essential for cell survival, and in multicellular organisms is implicated in the suppression of cancer. Cells have therefore evolved several pathways for repair of DNA damage [1]. While these repair pathways eliminate potentially cytotoxic and mutagenic changes to the genome, inefficient or inappropriate execution of repair can actually contribute to genome instability. One such process is genetic recombination, which is important for removal of certain DNA lesions and for faithful meiotic division. Recombination is initiated by the pairing of single-stranded DNA with a homologous double-stranded molecule, leading ultimately to DNA strand exchange [2]. Processes such as DNA replication and transcription, which generate regions of single-stranded DNA, may therefore inadvertently provide the substrate for initiation of recombination. It should not come as a surprise that mechanisms have evolved to ensure that recombination does not occur 'promiscuously' during these events. One such mechanism appears to involve the concerted action of RecQ helicases and topoisomerase III, and recent results have shed new light on how these two types of enzyme work together in the control of recombination.

Figure 1



RecQ and topoisomerase III (topo III) show a strand-passage activity. RecQ can enter and unwind a double-stranded plasmid; regions of single-stranded DNA thus created are stabilised by single-stranded DNA-binding protein (SSB). Topo III recognises this complex and passes a second plasmid through transient nicks in the single-stranded DNA. The reaction product is a full catenane. This model does not imply that RecQ/SSB necessarily interact with only one of the two (or more) plasmids to be catenated.

Escherichia coli RecQ is the prototypical member of a family of DNA helicases that has been highly conserved in evolution [3,4]. RecQ is required both for the initiation of the RecF pathway for homologous recombination [5] and for the suppression of 'illegitimate' recombination [6], which occurs between DNA segments containing little or no homology. There are at least five RecQ homologues in human cells, one of which, BLM, is mutated in the cancer-prone disorder Bloom's syndrome [7]. The *SGS1* gene encodes the sole RecQ homologue in budding yeast, and to date only one homologue, Rqh1, has been identified in fission yeast [8,9]. In addition to structural similarity, there is some conservation of function among these proteins, as expression of the *BLM* cDNA can functionally compensate in budding yeast cells for a lack of Sgs1 [10]. While the precise role of these helicases in eukaryotes is not known, mutants defective in *BLM*, *SGS1* or *rqh1*$^+$ display hyper-recombination [7,9,11].

Topoisomerases are another family of enzymes involved in regulating levels of genetic recombination [12]. Topoisomerases catalyse the passing of intact DNA molecules through transient DNA breaks — single-stranded breaks in the case of type I topoisomerases, and double-stranded breaks in the case of type II topoisomerases. These enzymes are essential for adjusting the level of DNA supercoiling during the operation of processes, such as replication, that involve the separation of complementary DNA strands by helicases; they are also required to remove knots and catenanes — links respectively within or between DNA molecules — from newly replicated chromosomes before cell division [13].

Budding yeast cells produce two type I topoisomerases, Top1 and Top3, and one type II topoisomerase, Top2. Mutation of *TOP1* or *TOP2* results in an increased rate of homologous recombination, but only at the ribosomal

(r)DNA locus [14]. Aside from this, *top1* mutants display a surprisingly mild phenotype. In contrast, *TOP2* is an essential gene, and *top2* mutants cannot complete mitosis, because of a defect in chromosome segregation [13]. Top1 and Top2 efficiently relax either negatively or positively supercoiled DNA, and the increased recombination seen in *top1* and *top2* mutants may be due to an accumulation of DNA torsional stress in the heavily transcribed rDNA [13]. Top3, however, can relax only negatively supercoiled DNA, and then only weakly [15]. Nevertheless, *top3* mutants display a reduced growth rate and hyper-recombination that affects repeat sequences throughout the genome [8,16]. In *E. coli*, there are two type I topoisomerases, topo I and topo III, encoded by the *topA* and *topB* genes, respectively. Both are structurally related to yeast Top3, and mutations in bacterial *topB* also result in hyper-recombination [17,18].

The hyper-recombination phenotype of mutants lacking RecQ helicases or Top3/topo III suggests that recombination is occurring promiscuously, and several lines of evidence indicate that RecQ helicases and Top3/topo III act in a common pathway to suppress this. In budding yeast, inactivation of Sgs1 reduces the level of hyper-recombination in *top3* mutants, and suppresses many of the other pleiotropic effects of *top3* mutations [8]. This genetic interaction suggests that the Sgs1 helicase acts upstream of Top3 to generate a DNA structure that is 'resolved' in some as yet undefined way by the strand-passage activity of Top3. Sgs1 and Top3 have also been shown to interact physically, which indicates that these enzymes probably function in a coordinated manner [8]. The recent demonstration [19] of a novel DNA-strand-passage activity catalysed by *E. coli* RecQ and topo III has provided the first direct biochemical evidence for a functional interaction between these two classes of proteins.

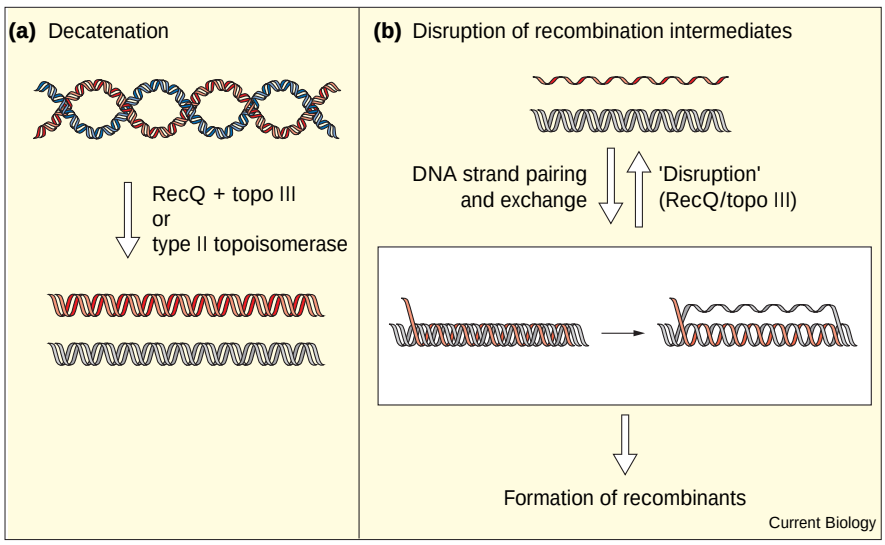
Using an assay system for DNA unwinding that relies on quantifying changes in DNA supercoiling induced by a translocating helicase, Harmon *et al.* [19] established that RecQ can unwind covalently closed plasmid DNA. This was a surprising result, given the usual requirement for a DNA end or an internal single-stranded DNA region for unwinding. The authors then asked if the product of this reaction could be a substrate for a catenation reaction catalysed by *E. coli* topo III. On its own, topo III efficiently catalyses the reverse reaction, that of unlinking catenated plasmids (decatenation), but only when one of the two DNA molecules contains a single-stranded DNA gap [20].

Harmon *et al.* [19] found that, in the presence of RecQ and single-stranded DNA-binding protein, topo III could catalyse the linking of covalently closed circular plasmids, forming catenanes (Figure 1). When the RecQ helicase activity was inhibited during the reaction, topo-III-mediated decatenation was also observed; this was still dependent upon the presence of RecQ [19]. Although Harmon *et al.* [19] failed to show a direct interaction between RecQ and topo III, neither Helicase II (UvrD) nor the presence of a single-stranded DNA gap in one of the plasmids was sufficient to achieve the same degree of topo-III-catalysed catenation [19]. These results suggest that RecQ does more than simply provide a single-stranded DNA-binding site for topo III, and that topo III must recognise a specific feature of the RecQ–DNA complex.

The strand-passage activity displayed by RecQ and topo III is essentially the reaction associated with a type II topoisomerase, and it may, therefore, have a role in chromosome segregation (Figure 2a). In budding yeast, *sgs1* mutants show elevated levels of chromosome non-disjunction during mitosis, suggestive of a defect in sister-chromatid

Figure 2

Models for potential functions of the strand-passage activity. (a) Decatenation. The unlinking of interlinked chromosomes by RecQ and topo III or a type II topoisomerase is required at late stages of replication. (b) Disruption of recombination intermediates. In homologous recombination, RecA catalyses the pairing of single-stranded DNA with a homologous double-stranded DNA. Depending upon when the invading strand becomes plectonemically wound, the strand-passage activity of RecQ/topo III could destroy the paired intermediate by restoring the parental strands (box, left). If recombination has proceeded further and the invading strand has already displaced its parental counterpart, RecQ/topo III may disrupt the heteroduplex DNA to permit re-annealing and re-linking of parental strands (box, right).



separation [21]. Moreover, in an *in vitro* replication system, bacterial topo III can catalyse decatenation of daughter molecules containing single-stranded DNA gaps [20]. Nevertheless, any potential role for RecQ and topo III in decatenation will need to be reconciled with the fact that neither *recQ* nor *topB* mutants of *E. coli* show obvious defects in chromosome partitioning. Sgs1 also interacts with Top2 and it may be the disruption of this complex that gives rise to the chromosome-segregation defects in *sgs1* mutant yeast cells [21].

The strand-passage activity described by Harmon *et al.* [19] might alternatively function in suppressing the promiscuous genetic recombination seen in RecQ helicase and topo III mutants. Recombination in *E. coli* is initiated by a region of single-stranded DNA bound by the RecA protein to form a nucleoprotein filament. This filament facilitates pairing with a homologous double-stranded DNA molecule, thereby promoting DNA-strand exchange [2]. Presently, it is unknown at what stage the invading strand becomes plectonemically wound around the homologous strand, an interlinking that is presumably topoisomerase-dependent. RecQ/topo III could act at an early step to disrupt recombination. For example, the inadvertent pairing of DNA strands over an extensive region, say between homoeologous — related but not identical — sequences or repeat sequences at different genomic locations, may require RecQ/topo III to unlink the mispaired strands. Alternatively, RecQ/topo III could act further downstream by unwinding the heteroduplex region formed during strand exchange, permitting re-annealing and re-linking of parental strands (Figure 2b).

Does this helicase–topoisomerase activity also occur in eukaryotes? Several pieces of evidence suggest that it does. The combined action of the two proteins described by Harmon *et al.* [19] was not restricted to bacterial proteins: yeast Top3 could substitute for *E. coli* topo III. An interaction between these two classes of enzymes has also been conserved in evolution, with Sgs1 acting in the same pathway as Top3 in budding yeast [8]. Genetic evidence exists for a similar interaction between Rqh1 and Top3 in fission yeast — deletion of $rqh1^+$ rescues the lethality of *top3* mutants (our unpublished observations). Moreover, BLM, the RecQ homologue mutated in Bloom's syndrome, forms a complex with topoisomerase III α, one of the two known human topoisomerase III isozymes (our unpublished observations). The hallmark of Bloom's syndrome is genome instability, which is almost certainly responsible for the increase in cancer incidence observed in affected individuals. Chromosomes from Bloom's syndrome cells show excessive sister-chromatid exchanges, which suggests a role for BLM in suppressing mitotic recombination [22]. The novel strand-passage activity of RecQ and topo III described by Harmon *et al.* [19] provides an insight into what this function might be, and raises the possibility of a role for topoisomerase III as a tumour suppressor in humans.

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